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Bacteriological Enumeration and Air Quality of Three Major Hospitals in Benin City, Nigeria

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ABSTRACT

*Airborne microorganisms results in nosocomial infections and are present in the air environment. Bacteriological air quality of hospital air environment is necessary to identify the airborne isolates responsible for these nosocomial infections. Samples were collected monthly from the designated sampling sites of three hospitals for a period of 12 months using the Settled Plate Methods on Nutrient Agar. The airborne bacterial colonies were characterized using the cultural, morphological and biochemical characteristics features. The mean airborne bacterial counts for Hospital 1 ranged from $2.35 \pm 1.36 \times 10^2$ cfu/m³ to $44.01 \pm 12.09 \times 10^2$ cfu/m³ that of Hospital 2 ranged from $2.09 \pm 0.45 \times 10^2$ cfu/m³ to $86.44 \pm 14.99 \times 10^2$ cfu/m³ while values for Hospital 3 ranged from $3.14 \pm 2.36 \times 10^2$ cfu/m³ to $94.29 \pm 7.86 \times 10^2$ cfu/m³. The evaluation of air quality revealed that hospital 3 had very high microbial load of 42.9% as compared to hospitals 1 and 2 with values of 24.1% and 27% respectively. The airborne bacterial isolates were characterized and identified to be *Staphylococcus* spp., *Bacillus cereus*, *Bacillus subtilis*, *Comamonas* spp, *Providencia* spp. and *Alcaligenes* spp. The Government owned hospitals had a higher microbial load than the other two privately-owned hospitals. More emphasis should be placed on this area of research because of the potential severity of the consequences of nosocomial infections. The Hospital managements, Ministry of Health, States and Federal Government, should establish a research database and set an acceptable range of isolated airborne flora in the hospital and other environments.*

1. Introduction

Air quality is an integral aspect of public health and the indoor air quality is largely determined by the quality of air from the outdoor, number and activities of humans in a particular environment as well as the ventilation methods that are available in that particular indoor environment [1]. The quality of these indoor air environment is not usually easy to control because microbiological air spores are always present in the air thereby exposing human occupants to risk of infection and contamination [2, 3]. The continual exposures to airborne microorganisms can lead to severe health effects such as respiratory disorder and hypersensitivity pneumonitis [4, 5]. Hospitals are places where sick or injured people go for medical examinations and treatments. Infected and non-infected

people are found in the hospital environment thus there is always an array of infectious microbes dominating the indoor air of hospitals leading to proliferation of virulent and antimicrobial resistant pathogens [6]. The patients, hospital workers and everyone associated in one way or the other to the hospital environments are affected by the hospital air [7]. Airborne pathogens have been reported to complicate patient's recovery rate from medical surgeries thereby leading to life-threatening infections [8, 9].

Airborne microorganisms that could result in nosocomial infections are present in the air, hence the need to study the microbiological air quality of the hospital air environment as airborne microorganisms are responsible for these nosocomial infections [10, 11, 12].

The physical, chemical and biological properties of the hospital indoor air can affect the health and wellbeing of patients, health workers and other persons in the environment [9]. Microorganisms are often transferred from the environment and infected individuals to objects which humans have contact with daily [13]. The sources of hospital airborne infection or contamination could be traced to a variety of factors, which includes the patient's own normal flora, linens, bed sheets, staff clothes and visitors. Activities such as sneezing, coughing, talking, yawning as well as the population of patients in a room or ward may also contribute to hospital acquired infections (HAI). Humans alters the microbial load of the space they occupy, building materials and equipment also influence the microbial composition [2, 7, 9, 14, 15]. The presence of few numbers of airborne microorganisms in indoor environments is a normal condition, but an increase of their concentration could represent a disease risk factor [16]. This study was aimed at assessing the bacteriological air quality of three major hospitals in Benin City.

2. Materials and Method

2.1 Study Sites and locations

The study was carried out in three highly patronized hospitals located in Benin City, Edo State, Nigeria. The first hospital was a privately owned and is located at Ekehuan road. The second was also privately owned and located at Airport road, while the third was a government owned hospital located at Sapele road, all in Benin City. Nine (9) designated units/wards namely; the Accident and Emergency ward, Male ward, Female ward, Children ward, Microbiology Laboratory, Maternity ward, Theatre, Treatment room and the Entrance Gate were used for the study.

2.2 Collection of sample

The airborne samples were collected monthly for a period of twelve (12) months; October, 2015 and September, 2016) from the designated sampling points using the Settled Plates Techniques. The freshly prepared Nutrient agar plates were exposed in triplicates at a height of 1 m above ground level for about 10 mins and incubated at 37 °C for 24h and up to 48 h for slow growing organisms [7, 17].

2.3 Bacterial enumeration and Characterization

The airborne bacterial isolates on the plates were enumerated and characterized using the cultural, morphological and biochemical characteristic features. The colony forming units (cfu) of the airborne bacterial flora were enumerated and expressed in cfu/m³ using the formula in Equation (1). The mean values from the triplicates plates were estimated and recorded. The air quality was evaluated based on the European Commission Sanitary Standards [18], [19], [20].

$$\text{cfu/m}^3 = \frac{a \times 10000}{p \times t \times 0.2} \quad (1)$$

where:

a: Number of colonies counted in Petri dish

p: Surface area of the 9cm diameter Petri dish (πr^2)

t: Time of exposure (10min)

2.4 Statistical Analysis of the Data

The means of the parameter (microbial counts) were compared against the monthly variation for each of the hospital sampled and all data were presented as mean \pm SEM (standard error) using one way Analysis of Variance (ANOVA) SPSS version 16. P values < 0.05 were considered statistically significant [21].

3. Results

The results obtained reveals that the mean airborne bacterial counts for Hospital 1 ranged from $2.35 \pm 1.36 \times 10^2$ cfu/m³ to $44.01 \pm 12.09 \times 10^2$ cfu/m³ (Fig. 1). Fig. 2 represents the mean airborne bacterial counts for Hospital 2 and values ranged $2.09 \pm 0.45 \times 10^2$ cfu/m³ to $86.44 \pm 14.99 \times 10^2$ cfu/m³. The mean airborne bacterial counts for Hospital 3 is represented in fig. 3 and the values ranged from $3.14 \pm 2.36 \times 10^2$ cfu/m³ to $94.29 \pm 7.86 \times 10^2$ cfu/m³.

Tables 1 to 3 shows the evaluation of air quality in the designated sampling points of the three hospitals studied based on the sanitary standards formulated by the European Commission. None of the designated sampling points across the three hospitals had very low (>50 cfu/m³) and low (50-100 cfu/m³) degree of contamination. In Table 1, the degree of contamination of the sampling points in hospital 1 had very high level (>2000 cfu/m³) of 24.1%, high level (between 500-2000 cfu/m³) of 64.8% and intermediate degree between (100-500 cfu/m³) of 11.1%. In hospital 2, the degree of contamination of the sampling points had very high level (>2000 cfu/m³) of 25.0%, high level (between 500-2000 cfu/m³) of 64.8% and intermediate degree (between 100-500 cfu/m³) of 10.2% (Table 2).

In Table 3, the degree of contamination of the sampling points in hospital 3 had very high level (>2000 cfu/m³) of 42.9%, high level (between 500-2000 cfu/m³) of 52.5% and intermediate degree between (100-500 cfu/m³) of 4.6%. The airborne bacterial isolates were characterized and identified to belong to the genus *Staphylococcus*, *Bacillus*, *Comamonas*, *Providencia* and *Alcaligenes* (Table 4).

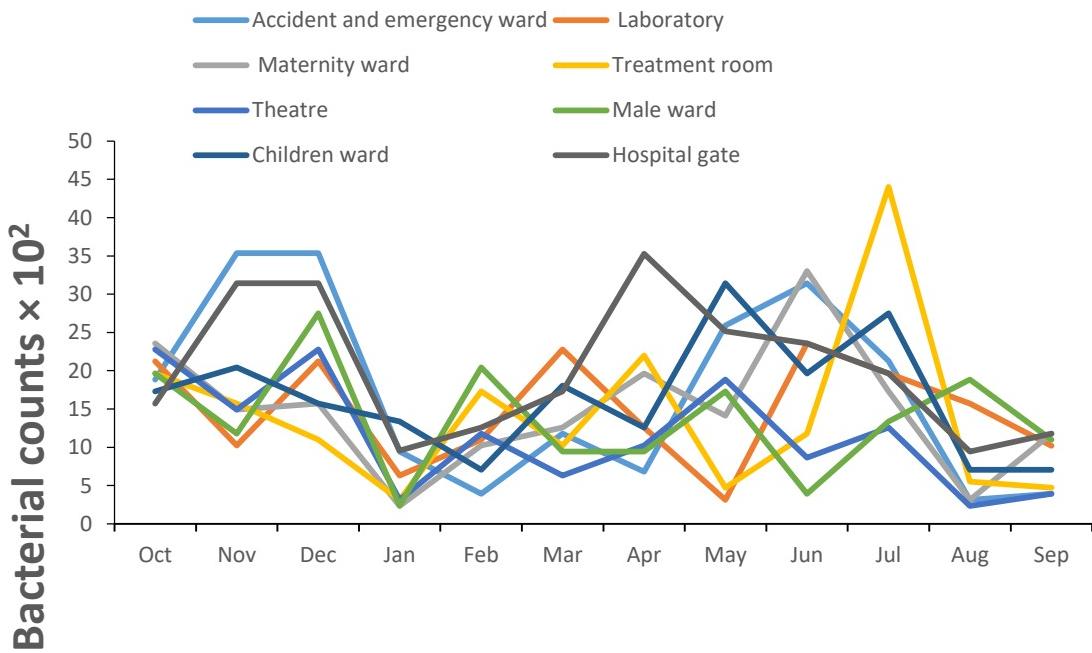


Fig. 1. Mean Airborne Bacterial Counts for Hospital 1 (Oct., 2015- Sept., 2016) in cfu/m³

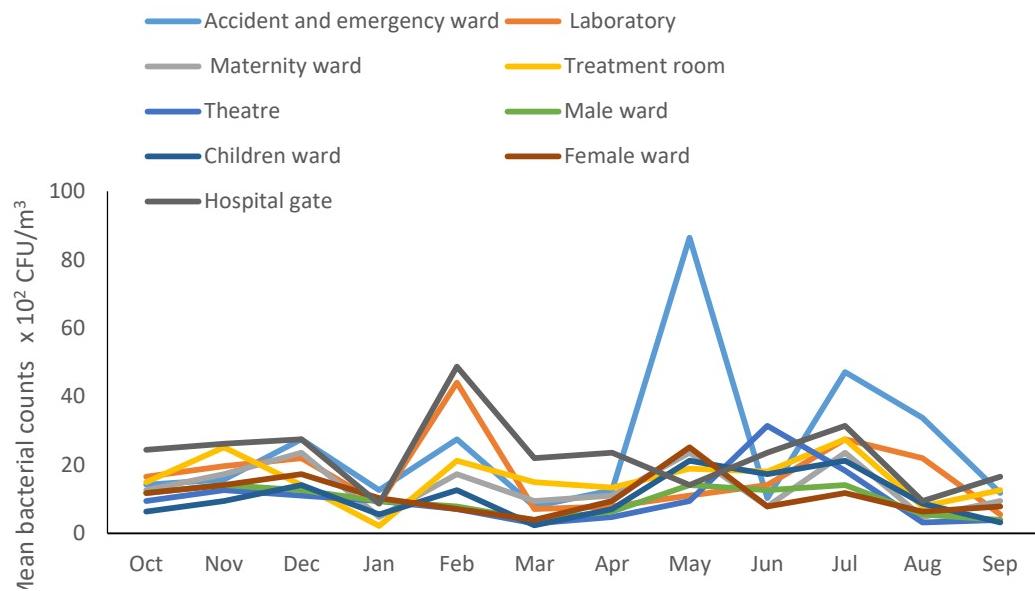


Fig. 2. Mean Airborne Bacterial Counts for Hospital 2 (Oct., 2015- Sept., 2016) in cfu/m³

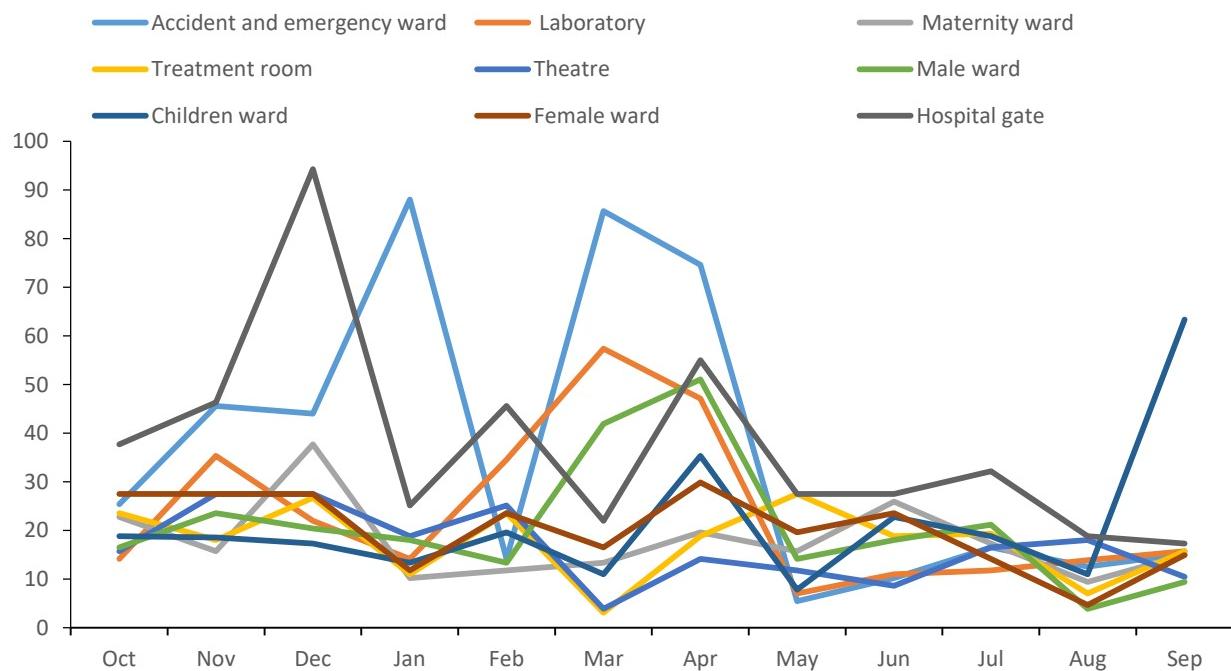


Fig. 3. Mean Airborne Bacterial Counts for Hospital 3 (Oct, 2015- Sept., 2016) in cfu/m³

Table 1: Evaluation of air quality in the designated sampling points at hospital 1 based on the sanitary standards formulated by the European commission

Months	Accident and emergency ward	Laboratory	Maternity room	Treatment room	Theatre	Male ward	Children ward	Female ward	Hospital gate
Oct	H	VH	VH	H	VH	H	H	H	H
Nov	VH	H	H	H	H	H	H	H	VH
Dec	VH	VH	H	H	VH	VH	H	H	VH
Jan	H	H	I	VH	I	I	H	H	H
Feb	I	H	H	H	H	VH	H	VH	H
Mar	H	VH	H	H	H	H	H	H	H
Apr	H	H	H	VH	H	H	VH	H	VH
May	VH	I	H	I	H	H	H	H	VH
Jun	VH	VH	VH	H	I	I	H	H	VH
Jul	VH	H	H	VH	H	H	VH	H	H
Aug	I	H	I	H	H	H	H	H	H
Sep	I	H	H	I	H	H	H	H	H

Key

Range Values (cfu/m ³)	Degree of contamination	Code	Contamination level (%)
< 50	very low	VL	-
50 – 100	Low	L	-
100-500	Intermediate	I	11(11.1)
500-2000	High	H	71(64.8)
>2000	Very high	VH	26(24.1)

TABLE 2: Evaluation of air quality in the designated sampling points at Hospital 2 based on the sanitary standards formulated by the European commission

Month s	Accident and emergency ward	Laboratory	Maternity room	Treatment room	Theatre	Male ward	Children ward	Female ward	Hospital gate
Oct	H	H	H	H	H	H	H	H	VH
Nov	H	H	H	VH	H	H	H	H	VH
Dec	VH	VH	VH	H	H	H	H	H	VH
Jan	H	H	I	I	H	H	I	H	H
Feb	VH	VH	H	VH	H	H	H	H	VH
Mar	H	H	H	H	I	H	H	H	VH
Apr	H	H	H	H	I	H	H	H	VH
May	H	H	VH	H	H	H	VH	VH	H
Jun	I	H	H	H	VH	H	H	H	VH
Jul	VH	VH	VH	VH	H	H	VH	H	VH
Aug	VH	VH	I	H	I	H	H	H	H
Sep	I	H	H	H	I	I	VH	H	H
Key									
Range Values (cfu/m ³)			Degree of contamination			Code	Contamination level (%)		
< 50			very low			VL	-		
50 – 100			Low			L	-		
100-500			Intermediate			I	10(10.2)		
500-2000			High			H	71(64.8)		
>2000			Very high			VH	27(25.0)		

TABLE 3: Evaluation of air quality in the designated sampling points at Hospital 3 based on the sanitary standards formulated by the European commission

Months	Accident and emergency ward	Laboratory	Maternity room	Treatment room	Theatre	Male ward	Children ward	Female ward	Hospital gate
Oct	VH	H	VH	VH	H	H	H	VH	VH
Nov	VH	VH	H	H	VH	VH	H	VH	VH
Dec	H	VH	VH	VH	VH	VH	H	VH	VH
Jan	H	H	H	H	H	H	H	H	VH
Feb	H	VH	H	VH	VH	H	VH	VH	VH
Mar	VH	VH	H	I	I	VH	VH	H	VH
Apr	H	VH	H	H	H	VH	VH	VH	VH
May	I	H	H	VH	H	H	VH	H	VH
Jun	H	H	VH	H	H	H	VH	VH	VH
Jul	H	H	H	H	H	H	VH	H	VH
Aug	H	H	H	H	H	I	H	I	H
Sep	H	H	H	H	H	H	VH	H	H
Key									

Range Values (cfu/m ³)	Degree of contamination	Code	Contamination level (%)
< 50	very low	VL	-
50 – 100	Low	L	-
100-500	Intermediate	I	5(4.6)
500-2000	High	H	58(52.5)
>2000	Very high	VH	45(42.9)

Table 4: Cultural and Biochemical Characteristics of the isolates

Cultural characteristics	A	B	C	D	E
Cell form	Circular	Circular	Circular	Circular	Circular
Colony margin	Entire	Flat	Undulate	Flat	Flat
Elevation	Convex	Flat	Flat	Flat	Slightly
Pigmentation	Cream	Cream	Cream	Cream	Cream
Optical characteristics	Opaque	Opaque	Opaque	Opaque	Opaque
Gram stain	+	-	-	-	+
Cell shape	Cocci	Rod	Rod	Rod	Rod
Capsule	None	+	None	None	None
Spores	None	None	None	None	None
BIOCHEMICAL					
Catalase	+	+	+	+	+
Oxidase	-	-	+	+	-
Indole	-	+	-	-	+
Urease	+	+	-	-	+
Methyl red	-	+	-	-	-
Voges Proskauer	-	-	-	-	+
Motility	-	-	+	+	+
Glucose	+	-	-	-	+
Lactose	+	-	-	-	+
Sucrose	+	-	-	-	+
Maltose	+	-	-	-	+
Probable organism	<i>Staphylococcus</i> spp.	<i>Providencia</i> spp.	<i>Alcaligenes</i> Spp.	<i>Comamonas</i> spp.	<i>Bacillus</i> spp.

3.1 Discussion

Microbial presence in the air is known to be a major attribute for infections and diseases. The microbiological air quality is an index of the hygienic status of a particular environment hence it is of great importance to study the quality of air we breathe. The number and type of airborne microorganisms play significant role in the degree of cleanliness of the environment especially in the health sector [22].

Microbial flora of indoor air depend on several factors including the number and hygienic standard of people present, the quality of occupational system and mechanical movement within the enclosed space. Human activities like talking, walking, laughing or crying and frequent movement of patient's relations and hospital staff in the hospital environment amongst other activities enhance the source(s) of indoor air contamination or pollution in the hospital environment.

This assertion was supported by previous investigations who opined that the presence of "people" is the most significant parameter resulting to elevation in bioaerosol counts in the absence of significant indoor sources [23]. Several countries have adopted indoor air quality standards based on

several factors deemed fit for safeguarding the health of citizens. However, following the sanitary standards stipulated by the European Commission, one would weep at the quality of the indoor air that the patients, patient's relations as well as hospital staff are exposed to in the three hospitals analyzed in this study.

Hospital 1, 2 and 3 had many wards with degree of contamination in the high ($500\text{-}2000 \text{ cfu}/\text{m}^3$) and very high range ($>2000 \text{ cfu}/\text{m}^3$). This correlated the findings of Knibbs et al. [24] who reported very high level of contamination in hospital setting and concluded that very high microbial load in hospital air pose serious health challenge to everyone in the hospital environment. Many researchers have confirmed that hospitals are sources of propagation and transmission of infections and this exposes patients, visitors and health workers to high risk. WHO [25] asserted that indoor air environments with a greater than $1000 \text{ cfu}/\text{m}^3$ microbial load should be considered contaminated. If this is carefully taken into consideration with values obtained from different wards in the three hospitals studied, it will be concluded that over 60% of the wards are unsafe and unfit for hospital staff, patients and patient's relations. WHO [25] also stated that a healthy indoor air or a safe clean air is a fundamental human right. From this perspective, one can assert that patients and staff are exposed to very high contaminated indoor environment and have been robbed of their fundamental right. The big question is by who? The sanitary standard set by World Health Organization is also in tandem with other sanitary standards reported in other countries where microbial thresholds ranging from 500 to $1000 \text{ cfu}/\text{m}^3$ is deemed as "acceptable" [26, 27].

Other authors such as Nevalainen and Morawska [28] and Cappitelli et al. [29] argued that $300 \text{ cfu}/\text{m}^3$ and $750 \text{ cfu}/\text{m}^3$ should be the limit for fungi and bacteria respectively. As at the time of this study, some of the wards were almost at their maximum capacity (such as the ever busy accident and emergency ward), with the influx of visitors in and out the wards. The high density of patients and staff as well as patient's relations could possibly increase the shading of bacteria and agitation of air as previously reported in the researches of Hospodsky et al. [30]; Qian et al. [31] and Meadow et al. [32].

The isolates identified are similar to those isolated by Ekhaise et al. [15] who isolated *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus* sp. with *Staphylococcus aureus* been the most frequently isolated. Rintala et al. [33] reported the high frequency of *Staphylococcus* sp. and *Micrococcus* sp. from office buildings. These are typical representatives of the normal flora of the human skin, oral cavity and outer ear. This emphasizes the impact of humans on the indoor air microbiota.

Majority of these bacteria have also been reported and implicated in cases of nosocomial infections defined as infections not present or incubating in a patient at the time of admission. Claudete et al. [11] reported that airborne microorganisms which are present in hospital environments are responsible for a number of these nosocomial infections. In advanced and developed world, there have been thousands of death due to nosocomial infections even with the hi-tech medical equipment and expertise. Recorded cases reveal that in the United States, the number of deaths due to nosocomial infections are about 99,000 yearly from 1.7 million nosocomial cases [34, 35].

The high level of contamination and bacterial load in the various wards observed in this study could be reduced by increase in the hygienic status of the hospital environments. There should be routine cleaning and disinfection of all wards in the hospitals. Also restriction should be made on the number of visitors that come to visit a patient. As observed across all sampling points, the theatre had the lowest microbial load and level of contamination. Theatres are places in the hospital that are fully restricted to visitors. This means that if the same is observed for all the wards and units in the hospital environment, microbial load can be restricted and reduced to the barest minimum. Previous

studies have also shown that humans alter the microbial load of an environment by their presence and activities hence the total or partial restriction of people not actually needed (like visitors) can help control the high level of contamination.

4. Conclusion

It was evident from the study that the Government owned Hospital had a higher microbial load than the other two privately owned hospitals with the Hospital gate having higher load than the other sampling areas. The Hospital managements, Ministry of Health, States and Federal Government, should establish a research database and set an acceptable range of isolated airborne flora in the hospital and other environments. The microbial load and type of isolates can be used in assessing and determining the level of cleanliness and air quality of a particular environment.

References

- [1] S.A. Awosika, F.A. Olajubu and N. A. Amusa (2012). Microbiological assessment of indoor air of a teaching hospital in Nigeria. *Asian Pacific Journal of Tropical Biomedicine*. Vol. 2(6):465-468.
- [2] A.A. Jaffal, I.M. Banet, A.A. EL Mogeth, H. Nsanze, A. Benar and A.S. Ameen (1997). Residential indoor airborne microbial populations in the United Arab Emirates. *Environmental International*. Vol. 23 (4):529-533.
- [3] F.O. Ekhaise and I.B. Ogboghodo (2011). Microbiological inddor and outdoor air quality of two major hospitals in Benin City, Nigeria. *Sierra Leone Journal of Biomedical Research*. Vol. 3(3): 169-174.
- [4] R.L. Gorny and J. Dutkiewicz (2002). Bacterial and fungal aerosols in indoor environment in Central and Eastern European Countries. *Annals of agricultural and Environmental medicine*. Vol. 9: 17-23.
- [5] M.F. Yassin and S. Almouqatea (2010). Assessment of airborne bacterial and fungi in an indoor and outdoor environment. *International Journal of Environmental Science and Technology*. Vol. 7(3):535-544.
- [6] J.A. Otter, J.L. Klein, T.L. Watts, A.M. Kearns and G.L. French (2007). Identification and control of an outbreak of Ciprofloxacin-susceptible EMRSA-15 on a neonatal unit. *Journal of Hospital Infection*. Vol. 67: 232-239.
- [7] F.O. Ekhaise, O.U. Ighosewe and O.D. Ajakpovi (2008). Hospital indoor airborne microflora in private and government owned hospitals in Benin City, Nigeria. *World Journal of Medical Sciences*. Vol. 3(1):19-23.
- [8] E. Hoseinzadeh, M.R. Samarghandi, S.A. Ghiasian, M.Y. Alikhani and G. Roshanaie (2013). Evaluation of bioaerosols in five educational hospital wards air in Hamedan, during 2011- 2012. *Jundishapur Journal of Microbiology*. Vol. 6(6):25-34.
- [9] D. Smith, J. Alverdy, G. An, M. Coleman, S. Garcia-Houchins, J. Green, K. Keegan, S.T. Kelley, B.C. Kirkup, L. Kociolek, H. Levin, E. Landon, P. Olsiewski, R. Knight, J. Siegel, S. Weber and J. Gilbert (2013). The Hospital Microbiome Project: Meeting Report for the 1st Hospital Microbiome Project Workshop on sampling design and building science measurements, Chicago, USA. *Standards in Genomic Science*. Vol. 8:112-117.
- [10] C.B. Beggs (2003). The airborne transmission of infection in hospital buildings: Fact or Fiction? *Indoor Built Environment*. Vol. 12: 9-18.
- [11] R.P. Claudete, V.L.J. Krebs, M.E. Aurer, L.S. Ruiz, F.E . Matsumoto, H.S. Elza, E.M.A. Dwiz and F.A.C. Vaz (2006). Nosocomial infection in newborns by *Pichia anomala* in Brazilian intensive care unit. *Medical Mycology*. Vol. 44: 479-484.
- [12] R.M. Badri, R.R. Alani and S.S. Hassan (2016). Identification and characterization of air bacteria from some schools of Baghdad City, Mesopotamia. *Environmental Journal*. Vol. 2(4): 9-13.
- [13] A.S. Abe, B. Inuwa, H. Abbas, A.M. Sule, H.A. Mohammed and M. Gero (2012). Identification and characterization of Bacteria air pathogens from homes in Zaria Metropolis. *International Journal of Science and Technology*. Vol. 2(7):443-447.
- [14] C. Alberti, A. Bouakline, P. Ribaud, C. Lacroix, P. Rousselot, T. Leblanc and T.O. Derovin (2001). Relationship between environmental fungal contamination and the incidence of invasive Aspergillosis in Hematology Patient. *Journal of Hospital Infection*. Vol. 48:198-206.
- [15] F.O. Ekhaise, E.E. Isitor, O. Idehen and A.O. Emoghene (2010). Airborne microflora in the atmosphere of an hospital environment of University of Benin Teaching Hospital (UBTH), Benin City, Nigeria. *World Journal of Agricultural Science*. Vol. 6(2):166-170.
- [16] J. Douwes, N. Pearce and D. Heederik (2003). Does environmental endotoxin exposure prevent asthma? *Thorax*. Vol. 57(1):86-90.

- [17] M. Augustowska and J. Dutkiewicz (2006). Variability of airborne microflora in a hospital ward within a period of one year. *Annals of Agriculture and Environmental Medicine*. Vol. 13:99-106.
- [18] R.E. Buchanan and N.E. Gibbons (1974). *Bergey's manual of determinative bacteriology*, 8th Edition. Williams and Wilkins, Baltimore. 1268pp.
- [19] M. Cheesbrough (2006). *Distinct Laboratory Practice in Tropical Countries*. Part 2. (2nd Ed). Cambridge University Press, New York, United States. 442pp.
- [20] [20] M. Stryjakowska-Sekulska, A. Piotraszewska-Pajak, A. Szyszka, M. Norwicki and M. Filipiak (2007). Microbiological quality of indoor air in University rooms. *Polish Journal of Environmental Studies*. Vol. 16(2): 624-632.
- [21] J. Isotalo (2001). *Basics of Statistics*. Finland: University of Tampere. 82pp.
- [22] C. Pasquarella, O. Pitzurra and A. Savino (2000). The index of microbial air contamination. *Journal of Hospital Infection*. Vol. 46:241 -256.
- [23] W.K. Jo and Y.J. Seo (2005). "Indoor and outdoor bioaerosol levels at recreation facilities, elementary schools, and homes". *Chemosphere*. Vol. 61(11): 1570-1579.
- [24] L. D. Knibbs, L. Morawska, S. C. Bell and P. Grzybowski (2011). Room ventilation and the risk of airborne infection transmission in 3 health care settings within a large teaching hospital. *American Journal of Infection Control*. Vol. 39(10):866-872.
- [25] WHO (2009). Guidelines for indoor air quality: dampness and mould. Copenhagen, Denmark. 228pp.
- [26] K. Naruka and J. Gaur (2013). Microbial air contamination in a school. *International Journal of Current Microbiology Applied Sciences*. Vol. 2(12): 404-410.
- [27] M. S. Kabir, F. Mridha, S. Islam and M. Sharifujjaman (2016). Microbiological pollutants in air and antibiotic resistance profile of some bacterial isolates. *Jahangirnagar University Journal of Biological science*. Vol. 5(1):47-56.
- [28] A. Nevalainen and L. Morawaska (2009). *Biological Agents in Indoor Environments. Assessment of Health Risks*. Work conducted by a WHO Expert Group between 2000 and 2003. WHO,
- [29] F. Cappitelli, P. Fermo, R. Vecchi, A. Piazzalunga, G. Valli, E. Zanardini and C. Sorlini (2009). Chemical-physical and microbiological measurements for indoor air quality assessment at the Ca' Granda historical archive, Milan (Italy). *Water, Air and Soil Pollution*. Vol. 201(1): 109–120.
- [30] D. Hspodsky, J. Qian, W.W. Nazaroff, N. Yamamoto, K. Bibby, H. Rismani-Yazdi and J. Peccia (2012). Human occupancy as a source of indoor airborne bacteria. *PloS ONE*. Vol. 7: e34867.
- [31] J. Qian, D. Hspodsky, N. Yamamoto, W.W. Nazaroff and J. Peccia (2012). Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom. *Indoor Air*. Vol. 22: 339–351.
- [32] J.F. Meadow, A.E. Altrichter, S.W. Kembel, J. Kline, G. Mhuireach, M. Moriyama, D. Northcutt, T.K. O'Connor, A.M. Womack, G.Z. Brown, J.L. Green and B.J.M. Bohannan (2013). Indoor airborne bacterial communities are influenced by ventilation, occupancy and outdoor air source. *Indoor Air*. Vol. 24:41 -48.
- [33] H. Rintala, M. Pitkaranta, M. Toivola, L. Paulin and A. Nevalainen (2008). Diversity and seasonal dynamics of bacterial community in indoor environment. *BioMedCentral Microbiology*. Vol. 8(56):1471-1490.
- [34] S.K. Jain, M.A. Pass, K.M. Murphy, J.M. Pisciotta, P.F. Scholl, J.F. Casella, D.J. Sullivan, D. Persaud and T.M. Perl (2005). "Nosocomial malaria and saline flush". *Emerging Infectious Diseases Journal*. Vol. 11(7): 1097-1099.
- [35] R.M. Klevens, J.R. Edwards, C.C. Richards, T.C. Horan, R.P. Gaynes, D.A. Pollock and D.M. Cardo (2007). "Estimating health care-associated infections and deaths in US Hospitals. *Public Health Reports*. Vol. 112(2): 160-166.